REMARKS

Status of the Claims

Following this Amendment, claims 1-4, 7-14, 17-21, 23, 25-41, 45-47, 49-50, 52-61, 207-212, and 218-242 are now pending in the application. In the present Amendment, claims 22, 24, 42-44, 48, 51 and 213-217 have been canceled, claims 1-4, 7-8, 11, 13-14, 17-24, 36-59, 207-208, and 210-226 have been amended and new claims 227-242 have been added. Support for these new and amended claims can be found throughout the specification and the originally filed claims. Applicants have not introduced any new matter by the amendments.

Specifically, support for bacterial and yeast glucosamine-6-phosphate synthases can be found, *inter alia*, at Example 2. Support for bacterial and yeast glucosamine-6-phosphate acetyltransferases can be found, *inter alia*, at Example 13. Support for increased expression of a bacterial or yeast glucosamine-6-phosphate acetyltransferase can be found at page 29, lines 5-27, and at Examples 13 and 14.

In addition, support for a the bacterial or yeast glucosamine-6-phosphate synthase that has at least one amino acid substitution that reduces product inhibition of the glucosamine-6-phosphate synthase as compared to the wild-type glucosamine-6-phosphate synthase, and a naturally occurring bacterial or yeast glucosamine-6-phosphate synthase that has less product inhibition than the endogenous glucosamine-6-phosphate synthase can be found at page 28, line 6, to page 29, line 4, and at Examples 3 and 4.

Support for the deletion or inactivation of N-acetylglucosamine-6-phosphate deacetylase, glucosamine-6-phosphate deaminase, and N-acetyl-glucosamine-specific

enzyme II^{Nag} (*E. coli nagA*, *nagB*, and *nagE*), mannose transporter EIIM,P/III^{Man} (*E. coli manXYZ*) and phosphofructokinase, and an enzyme involved in glycogen synthesis can be found, *inter alia*, at Examples 15, 14, 22 and 27, respectively.

Claim Objections

Claim 58 was objected to due to the recitation of the term "and" between items c) and d) rather than between items d) and e). Claim 58 has been amended so that the term "and" and a semicolon now appear between the last two listed items, as suggested by the Examiner.

Claim 207 was objected to due to the recitation of "inducing the transcription of the nucleic acid sequence." Claim 207 has been amended to indicate that the induction applies to the nucleic acid rather than the sequence, as suggested by the Examiner.

Accordingly, Applicants respectfully request that these objections be withdrawn.

Rejections Under 35 U.S.C. § 112, Second Paragraph

Claims 7, 17, 213-217, 219-221 and 223-226 were rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for directly or indirectly reciting the term "at least about." Claims 213-217 have been canceled. Claims 7, 17 and 219 (Claims 220-221 and 223-226 dependent thereon) have been amended to remove the term "about," as suggested by the Examiner.

Claim 58 was rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for reciting a step of recovering a product. As suggested, by the Examiner, this step has been deleted to clarify the claim language.

Claim 59 was rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for lack of an antecedent basis for step (e). Claim 59 has been amended to properly depend from Claim 58.

Claim 207 (and claims 208-212 dependent thereon) were rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for reciting the term "after step (ii)." Claim 207 has been amended to remove the phrase "after step (ii)" and to clarify that, as described in the specification, it is the addition of lactose (independent of the presence or absence of glucose) that induces transcription. Further, step (iii) may be carried out by fermenting the bacterium or yeast in the same medium as step (ii) (which may contain residual glucose from step (i)), or by adding more glucose to the medium at step (iii). Claim 207 is not limited by the completion of the induction process. Rather, step (iii) is merely carried out at some time after step (ii).

Claim 208 was rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for reciting "step (iii) of fermenting." Claim 208 has been amended to read "step (iii)," as suggested by the Examiner.

Claim 210 was rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for reciting the term "step (ii)." As suggested by the Examiner, Claim 210 has been amended to recite "step (i)."

Accordingly, in light of the amendments discussed above, Applicants respectfully request that all rejections under 35 U.S.C. § 112, second paragraph, be withdrawn.

Rejections Under 35 U.S.C. § 112, First Paragraph

Written Description

Claims 46, 50, 53 and 220-222 were rejected under 35 U.S.C. § 112, first paragraph, as failing to comply with the written description requirement. According to the Office, the term "an amino acid sequence of SEQ ID NO:X" can be interpreted as "an amino acid within SEQ ID NO:X." Claims 7-8, 17-18, 20, 46, 50, 53 and 220-222 have been amended to recite "the amino acid sequence of SEQ ID NO:X," as suggested by the Examiner. Applicants thus request that these rejections be withdrawn.

Claims 1-4, 7-14, 17-61, and 207-226 were also rejected under 35 U.S.C. § 112, first paragraph, as failing to comply with the written description requirement. According to the Office, these claims encompass, but the specification does not support, (1) any genetic modification that would increase or decrease the activity of a genus of enzymes, (2) a genus of nucleic acids encoding glucosamine-6-phosphate synthases and glucosamine-6-phosphate acetyltransferases, and (3) nucleic acids encoding proteins comprising any fragment of SEQ ID NOS: 6 and 30. Office Action, pages 7-8.

Applicants respectfully traverse this rejection. The written description requirement is satisfied if the specification discloses the invention in sufficient detail to allow a person skilled in the art to reasonably conclude that the inventor had possession of the invention as claimed. M.P.E.P. § 2163. While claims drawn to a genus may be adequately supported by the disclosure of a representative number of species within the genus, the Federal Circuit has made clear that the specification need not describe every

permutation of an invention nor subject matter known to those of skill in the art. *Capon v. Eshhar,* 418 F.3d 1349,1359-60 (Fed. Cir. 2005).

In the present specification, Applicants have specifically exemplified in detail the sequences of bacterial, yeast and plant glucosamine-6-phosphate synthases and glucosamine-6-phosphate acetyltransferases, and have taught that the invention is not limited to enzymes of a particular species or genus. Rather, the specification demonstrates that the methodology is applicable to glucosamine-6-phosphate synthases and glucosamine-6-phosphate acetyltransferases from organisms as diverse as bacteria, yeast and higher plants such as *Arabidopsis thaliana*, since these and other organisms have similar amino sugar metabolic pathways and genes and proteins having similar structure and function within such pathways. Moreover, Applicants have demonstrated that many other glucosamine-6-phosphate synthases and glucosamine-6-phosphate acetyltransferases were known in the art at the time of the invention. Consistent with Federal Circuit precedent, Applicants are not required to provide the sequences of every glucosamine-6-phosphate synthase and glucosamine-6-phosphate acetyltransferase suitable for use in the present invention, since these sequences were already known in the art.

Further, it is well known in the art that similar enzymes from different organisms may possess low overall amino acid identity, yet contain significant homology or identity in domains important for enzymatic activity. Applicants specifically disclose such areas of homology. For instance, in Example 13, Applicants demonstrate the conserved structural regions of glucosamine-6-phosphate acetyltransferases from *E. coli*, *S. cerevisiae*, *C. albicans* and *A. thaliana*. Applicants then specifically show that even

though glucosamine-6-phosphate acetyltransferases from *S. cerevisiae* and *A. thaliana* share only 38.9% amino acid identity, they both catalyze the same reaction when expressed in *E. coli* cells. Thus, Applicants have clearly demonstrated that a genus of glucosamine-6-phosphate synthases and glucosamine-6-phosphate acetyltransferases from diverse organisms are suitable for use in the present invention, despite lacking a high degree of structural identity. Because these enzymes were known in the art at the time of the invention, Applicants have demonstrated possession of the full scope of the claimed invention.

Applicants respectfully submit that the specification, in combination with the knowledge in the art, provides adequate support for the instant claims. The specification provides numerous examples of genetic modifications, nucleic acids and proteins suitable for use with the invention. Moreover, additional examples of species within these genera are known in the art. The specification thus provides sufficient detail to allow one of skill in the art to conclude that Applicants invented the full scope of the claimed invention.

However, in an effort to expedite prosecution, Applicants have amended the claims and submit that the specification provides adequate support for the claims as amended. The specification provides specific examples of the culturing of bacteria and yeast that comprise at least one genetic modification, as recited in the claims. Likewise, specific examples of genetic modifications resulting in increased gene expression, inactivation of a gene and reduced product inhibition of glucosamine-6-phosphate synthase activity are disclosed. Moreover, the specification exemplifies several nucleic acids encoding bacterial and yeast glucosamine-6-phosphate synthases and

glucosamine-6-phosphate acetyltransferases (see, e.g., Examples 2-4 and 13), as recited in the claims.

Finally, as discussed above, Claims 7-8, 17-18, 20, 46, 50, 53 and 220-222 have been amended to recite "the amino acid sequence of SEQ ID NO:X." The claims also recite amino acid sequences that are 95% identical to sequences disclosed in the specification and further retain the enzymatic activity of the recited enzyme. The specification and claims place a clear limit on the number of amino acid modifications that may be made to the recited sequences, because one of skill in the art is readily apprised of the number of changes that constitute 5% of the amino acid residues of these sequences. An assay is also disclosed for identifying activity of the enzymes. The disclosed species of sequences are representative of the genus because all members have at least 95% structural identity with the reference protein and because of the disclosure of an assay which applicant provided for identifying all of the at least 95% identical variants of the recited sequences which are capable of the specified enzyme activity. Moreover, multiple enzymes with less than 95% identity to one another are disclosed and shown to be operable in the invention.

Therefore, in view of the amendments and explanations provided above, Applicants respectfully submit that the specification provides adequate written description support for all pending claims. Accordingly, Applicants request that all rejections under 35 U.S.C. § 112, first paragraph, be withdrawn.

Enablement

Claims 1-4, 7-14, 17-61, and 207-226 were rejected under 35 U.S.C. § 112, first paragraph, as failing to comply with the enablement requirement. According to the

Office, the specification does not enable one of skill in the art to make and use the invention as claimed. Office Action, page 11. Specifically, the Office asserts that the specification does not provide enabling support for culturing a microorganism that has any genetic modification that would increase or decrease the activity of an enzyme involved in glucosamine/N-acetylglucosamine synthesis or would result in the inactivation of phosphofructokinase or an enzyme involved in glycogen synthesis. Applicants respectfully traverse.

To satisfy the enablement requirement, the specification must contain sufficient disclosure to enable one skilled in the art to make and use the claimed invention without undue experimentation. M.P.E.P. § 2164. A determination of whether the claims are enabled thus involves, *inter alia*, the level of skill in the art and the amount of direction provided by the inventor. *In re Wands*, 858 F.2d 731, 737 (Fed. Cir. 1988). Applicants submit that the specification provides sufficient disclosure to allow one of skill in the art to practice the full scope of the claims.

As discussed above, the claims have been amended to recite the culturing in a fermentation medium of a bacterium or yeast. The specification provides specific examples of the culturing of bacteria and yeast that comprise at least one genetic modification, as recited in the claims. (see, e.g., Examples 2 and 20, respectively). Methods of culturing strains of bacteria and yeast, as well as the expression of modified genes within the cultured strains of bacteria and yeast were well known in the art as of the priority date of the instant application.

The specification also discloses specific examples of genetic modifications resulting in increased gene expression or inactivation of enzymes involved in

glucosamine/N-acetylglucosamine synthesis, phosphofructokinase, and enzymes involved in glycogen synthesis, as recited in the amended claims. In addition, methods of increasing gene expression and inactivating or deleting genes in bacteria and yeast are well known in the art. Further, the enzymatic pathways leading to glucosamine, N-acetylglucosamine and glycogen synthesis in bacteria and yeast are disclosed in the specification and/or well known to one skilled in the art.

Applicants specifically describe techniques for making and screening bacteria and yeast strains that possess at least one mutation or deletion in the amino acid sequence of the glucosamine-6-phosphate synthase that results in a reduced product inhibition of the glucosamine-6-phosphate synthase activity as compared to the wild-type glucosamine-6-phosphate synthase, and disclose at least six strains that possess this mutation. The specification makes clear that it is not necessary to know where to modify the sequence in order to produce the recited microorganisms and use them in the claimed invention. However, if one wishes to determine the identity of the mutation after the microorganism is identified, this may be accomplish by routine sequencing.

Finally, the amended claims recite the partial or complete deletion of phosphofructokinase or enzymes involved in glycogen synthesis. The specification describes the complete deletion of genes encoding these enzymes (see, e.g., Examples 22 and 27). One of skill in the art, using routine techniques well known in the art, can adapt this disclosure to disrupt these enzymes' activities by partially deleting the same genes. Therefore, the specification provides enabling support for the partial or complete deletion of genes encoding phosphofructokinase or enzymes involved in glycogen synthesis.

Combining the teachings of the specification with the knowledge in the art at the priority date of the application, one of skill in the art could readily make and use the full scope of the claimed invention without undue experimentation. Therefore, the enablement requirement of 35 U.S.C. § 112, first paragraph, has been satisfied. Applicants thus respectfully request that these rejections by withdrawn.

Conclusions

In view of the foregoing amendments and remarks, Applicants respectfully request reconsideration and reexamination of this application and the timely allowance of the pending claims. If the Examiner has any questions regarding this Amendment and Response, the Examiner is invited to contact the undersigned at 303-863-9700.

The required three-month extension of time fee of $1020.00 is submitted herewith via EFS-Web. In the event that additional fees are due in connection with this response, please debit Deposit Account No. 19-1970.

Respectfully submitted,

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